

Figure 2

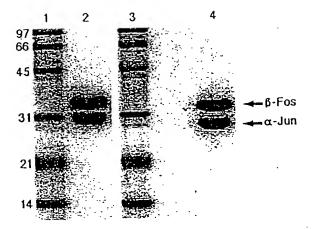
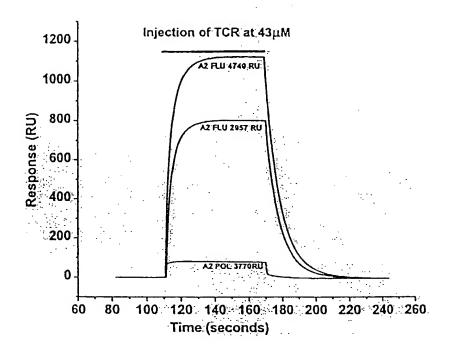


Figure 3



Α

Poly-C 'anchor primer':

В

TCR α chain constant region specific primer:

Xma I 5'- ATA TAA <u>OOC GOG</u> GAA OOA GAT OOC CAC AGG AAC TIT CTG GOC TGG GGA -3'

C

TCR β chain constant region specific primer:

 $$\rm Xma\ I$$ 5'- ATA TAA $\rm \underline{\rm CCC}$ GGG GAA CCA GAT CCC CAC AGT CTG CTC TAC CCC AGG CC -3'

```
Figure 5
```

A

c-jun 5' primer:

Xma I

5'- CATACACCCGGGGGTAGAATCGCCCGGCTGGAG -3'

B

c-jun 3' primer:

Xho I

5' - GIGIGIG<u>CTCGAG</u>GATCCTAGTAGTTCATGACTTTCIGTTTAAGCIGIGC -3'

Bam HI

c-fos 5' primer:

Xma I

5' -CATACACCCGGGGTCTGACTGATACACTCCAAGCGGAG -3'

D

c-fos 3' primer:

Xho I

5'- TGTGTGCTCGAGGATCCTAGTAAGCTGCCAGGATGAACTCTAGTTTTTC -3'

Bam HI

Figure 6.

4

909 CTG GAG TCG K A Q N AAA GCT CAG AAC ACC TTG Н GTG AAA L E E K CTG GAG GAA AAA gcc Bcc 5'- AGA ATC

-3, V M N GTC ATG AAC S T A N M L R E Q V A Q L K Q K TCC ACG GCC AAC ATG CTC AGG GAA CAG GTG GCA CAG CTT AAA CAG AAA

C-jun leucine zipper DNA and amino acid (one-letter code) sequences as fused to TCR alfa chains.

В

Q CAG TTG GCT AAG E GAG D GAT GAA 禸 CTA CAA GAC GAG ⊡ gce CAA CIC D GAT 5′ –

-3, GCA CIG F I TTC ATC (L L K E K E K L E CTG CTG AAG GAA AAA CTA GAG T E I A N ACC GAG ATT GCC AAC

C-fos leucine zipper DNA and amino acid (one-letter code) sequences as fused to TCR beta chains.

r

Figure 7

A

Mutation of cysteine to serine, forwards (sense) primer, indicating amino acid sequence and the mutation:

C ↓ D S R Y S L S S 5'- GAC TOC AGA TAC AGC CTG AGC AGC CG -3'

B

Mutation of cysteine to serine, backwards (nonsense) primer:

5'- CG GCT GCT CAG GCT GTA TCT GGA GTC -3'

\mathbf{C}

Mutation of cysteine to alanine, forwards (sense) primer, indicating amino acid sequence and the mutation:

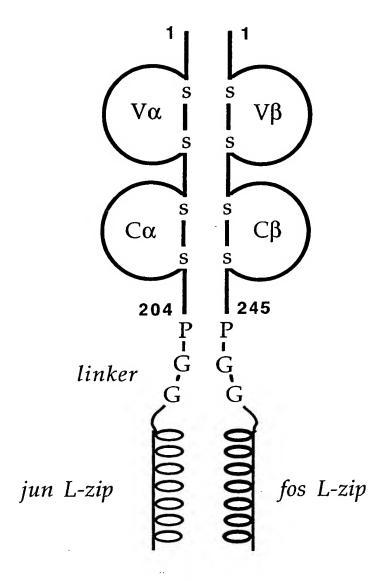
C ↓ D S R Y A L S S 5'- GAC TCC AGA TAC GCT CIG AGC AGC CG -3'

D

Mutation of cysteine to alanine, backwards (nonsense) primer:

5'- CG GCT GCT CAG AGC GIA TCT GGA GTC -3'

Figure 8



A

 5^{\prime} PCR primer for the human $V\alpha10.2$ chain of the JM22 Influenza Matrix peptide-HLA-A0201 restricted TCR:

M Q L L E Q S P Q F L 5'- gctctaga<u>cat ATG</u> CAa CTa CTa GAa CAa AGt CCT CAG TTT CTA Nde I

S I Q E AGC ATC CAA GAG G -3'

В

5' PCR primer for the human $V\beta17$ chain of the JM22 Influenza Matrix peptide-HLA-A0201 restricted TCR:

M V D G G I T Q S 5'- gctctaga<u>cat ATG</u> GIG GAT GGT GGA ATC ACT CAG TCC C -3' Nde I

5' PCR primer for the mouse $V\alpha 4$ chain of the Influenza nucleoprotein peptide-H2-D^b restricted TCR:

M D S V T Q M Q G Q V 5'- gctctaga<u>cat ATG</u> GAt TCt GTt ACt CAa ATG CAa GGt CAa GTG Nde I

T L S S ACC CTC TCA TCA G -3' Figure 9 (continued)

D

5' PCR primer for the mouse $V\beta11$ chain of the Influenza nucleoprotein peptide-H2-D^b restricted TCR:

M E P T N A G V I Q 5'- gctctaga<u>cat ATG</u> GAa CCa ACa AAt GCt GGt GTt ATC CAA

T P R H ACA CCT AGG CAC -3'

E

5' PCR primer for the human $V\alpha23$ chain of the 003 HIV-1 Gag peptide-HLA-A0201 restricted TCR:

M K Q E V T Q I 5'- ggaattc<u>cat atg</u> AAA CAa GAG GTt ACa CAa ATT CC -3' Nde I

F

5' PCR primer for the human V β 5.1 chain of the 003 HIV-1 Gag peptide-HLA-A0201 restricted TCR:

M K A G V T Q T 5'- ggaattc<u>cat atq</u> AAa GCT GGA GTt ACT CAA ACT CC -3'

Figure 9 (continued)

G

5' PCR primer for the human $V\alpha 2.3$ chain of the HTLV-1 Tax peptide-HLA-A0201 restricted A6 TCR:

M Q K E V E Q K 5'-ccccc cat ATG CAG AAG GAA GTG GAG CAG AAC -3' Nde I

 \mathbf{H}

5' PCR primer for the human V β 12.3 chain of the HTLV-1 Tax peptide-HLA-A0201 restricted A6 TCR:

 $$\rm M$ K A G V T Q T $$\rm 5'-$ ccccc cat ATG AAC GCT GGT GTC ACT CAG ACC -3' Nde I

T

5' PCR primer for the human $V\alpha17.2$ chain of the HTLV-1 Tax peptide-HLA-A0201 restricted B7 TCR:

M Q Q K N D D Q Q V 5'-ccccc <u>cat ATG</u> CAA CAa AAa AAT GAT GAC CAG CAA GTT Nde I

K Q N AAG CAA AAT -3' Figure 9 (continued)

5' PCR primer for the human Vβ12.3 chain of the HTLV-1 Tax peptide-HLA-A0201 restricted B7 TCR:

M N A G V T Q T P K F 5'-ccccc cat ATG AAC GCT GGT GTC ACT CAG ACC CCA AAA TTC Nde I

Q CAG -3'

K 3' PCR primer for human $C\alpha$ chains, generally applicable:

5'- cataca <u>ccc ggg</u> GGA ACT TIC TGG GCT GGG GAA GAA GG -3' Xma I

L 3' PCR primer for human C β chains, generally applicable:

5'- cataca <u>ccc ggg</u> GTC TGC TCT ACC CCA GGC CTC -3'
Xma I

TCR alfa>
M Q L L E Q S P Q F L S I Q E G E N L T
ATGCAaCTaCTaGAaCAAGtCCTCAGTTTCTAAGCATCCAAGAGGGAGAAAATCTCACT

L T F Q F G D A R K D S S L H I T A A Q CTAACCTTTCAGTTTGGTGATGCAAGAAAGGACAGTTCTCTCCACATCACTGCGGCCCAG

P G D T G L Y L C A G A G S Q G N L I F CCTGGTGATACAGGCCTCTACCTCTGTGCAGGAGCGAAGCCAAGGAAATCTCATCTTT

Q L R D S K S S D K S V C L F T D F D S CAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTGATTCT

Q T N V S Q S K D S D V Y I T D K T V L CAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAACTGTGCTA

D M R S M D F K S N S A V A W S N K S D GACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAATCTGAC

F A C A N A F N N S I I P E D T F F P S TTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGC

<TCR alfa <u>linker</u> c-jun>

PESSPGGRIARLEEKVKTLKCCAGAAAGTTCCcccgggGGTAGAATCGCCCGGCTGGAGGAAAAAGTGAAAACCTTGAAA

A Q N S E L A S T A N M L R E Q V A Q L GCTCAGAACTCGGAGCTGCCTCCACGCCAACATGCTCAGGGAACAGGTGGCACAGCTT

K Q K V M N Y *
AAACAGAAAGTCATGAACTACTAG

TCR beta>

V T L S C E Q N L N H D A M Y W Y R Q D GTGACCCTGAGTTGTGAACAGAATTTGAACCACGATGCCATGTACTGGTACCGACAGGAC

P G Q G L R L I Y Y S Q I V N D F Q K G CCAGGGCAAGGGCTGAGATTGATCTACTACTCACAGATAGTAAATGACTTTCAGAAAGGA

D I A E G Y S V S R E K K E S F P L T V GATATAGCTGAAGGGTACAGCGTCTCTCGGGAGAAGAAGGAATCCTTTCCTCTCACTGTG

T S A Q K N P T A F Y L C A S S S R S SQ ACATCGGCCCAAAAGAACCCGACAGCTTTCTATCTCTGTGCCAGTAGTTCGAGGAGCTCC

Y E Q Y F G P G T R L T V T E D L K N V TACGAGCAGTACTTCGGGCCGGGCACCAGGCTCACGGTCACAGAGGACCTGAAAAACGTT

F P P E V A V F E P S E A E I S H T Q K
TTCCCACCGAGGTCGCTGTGTTTGAACCATCAGAAGCAGAGATCTCCCACACCCAAAAG

A T L V C L A T G F Y P D H V E L S W W GCCACACTGGTGGCCTGCCCACAGGCTTCTACCCCGACCACGTGGAGCTGAGCTGGTGG

V N G K E V H S G V S T D P Q P L K E Q GTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACAGACCCGCAGCCCCTCAAGGAGCAG

PALNDSRYCLSSRLRVSATFCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCTTC

W Q N P R N H F R C Q V Q F Y G L S E N TGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGGAGAAT

D E W T Q D R A K P V T Q I V S A E A W GACGAGTGGACCCAGGATAGGGCCAAACCTGTCACCCAGATCGTCAGCGCCGAGGCCTGG

<TCR beta <u>linker</u> c-fos>

G R A D P G G L T D T L Q A E T D Q L E GGTAGAGCAGACcccgggGGTCTGACTGATACACTCCAAGCGGAGACAGATCAACTTGAA

D K K S A L Q T E I A N L L K E K E K L GACAAGAAGTCTGCGTTGCAGACCGAGATTGCCAATCTACTGAAAGAAGAAGAAAAACTA

E F I L A A Y *
GAGTTCATCCTGGCAGCTTACTAG

TCR alfa> M N Y S P A L V T V M L F V F G R T H G ATGAACTATTCTCCAGCTTTAGTGACTGTGATGCTGTTTGTGTTTGGGAGGACCCATGGA

DSVTQMQGQVTLSEDDFLFI GACTCAGTAACCCAGATGCAAGGTCAAGTGACCCTCTCAGAAGACGACTTCCTATTTATA

N C T Y S T T W Y P T L F W Y V Q Y P G AACTGTACTTATTCAACCACATGGTACCCGACTCTTTTCTGGTATGTCCAATATCCTGGA

E G P Q L L L K V T T A N N K G I S R G GAAGGTCCACAGCTCCTTTTGAAAGTCACAACAGCCAACAACAAGGGAATCAGCAGAGGT

FEATYDKGTTSFHLQKASVQTTTGAAGCTACATATGATAAAGGAACAACGTCCTTCCACTTGCAGAAAGCCTCAGTGCAG

E S D S A V Y Y C V L G D R Q G G R A L GAGTCAGACTCTGCTGTGTACTACTGTGTGCTGGGTGATCGACAGGGAGGCAGAGCTCTG

IFGTGTTVSVSPNIQNPEPA

ATATTTGGAACAGGAACCACGGTATCAGTCAGCCCCAACATCCAGAACCCAGAACCTGCT

V Y Q L K D P R S Q D S T L C L F T D F GTGTACCAGTTAAAAGATCCTCGGTCTCAGGACAGCACCCTCTGCCTGTTCACCGACTTT

D S Q I N V P K T M E S G T F I T D K T GACTCCCAAATCAATGTGCCGAAAACCATGGAATCTGGAACGTTCATCACTGACAAAACT

V L D M K A M D S K S N G A I A W S N Q GTGCTGGACATGAAAGCTATGGATTCCAAGAGCAATGGGGCCATTGCCTGGAGCAACCAG

T S F T C Q D I S K E T N A T Y P S S D ACAAGCTTCACCTGCCAAGATATCTCCAAAGAGACCAACGCCACCTACCCCAGTTCAGAC

<TCR alfa linker c-jun>
V P G G R I A R L E E K V K T L K A Q N
GTTcccgggGGTAGAATCGCCCGGCTGGAGGAAAAAGTGAAAACCTTGAAAGCTCAGAAC

S E L A S T A N M L R E Q V A Q L K Q K TCGGAGCTGGCGTCCACGGCCAACATGCTCAGGGAACAGGTGGCACAGCTTAAACAGAAA

V M N Y *
GTCATGAACTACTAG

TCR beta>
M K A G V T Q T P R Y L I K T R G Q Q V
ATGAAAGCTGGAGTTACTCAAAACTCCAAGATATCTGATCAAAACGAGAGGACAGCAAGTG

T L S C S P I S G H R S V S W Y Q Q T P ACACTGAGCTGCTCCCCTATCTCTGGGCATAGGAGTGTATCCTGGTACCAACAGACCCCA

G Q G L Q F L F E Y F S E T Q R N K G N GGACAGGGCCTTCAGTTCCTCTTTGAATACTTCAGTGAGACACAGAGAAACAAAGGAAAC

FPGRFSGRQFSNSRSEMNVS TTCCCTGGTCGATTCTCAGGGCGCCAGTTCTCTAACTCTCGCTCTGAGATGAATGTGAGC

T L E L G D S A L Y L C A S S F D S G N ACCTTGGGGGGACTCGGCCCTTTATCTTTGCGCCAGCAGCTTCGACAGCGGGAAT

S P L H F G N G T R L T V T E D L N K V TCACCCCTCCACTTTGGGAACGGGACCAGGCTCACTGTGACAGAGGACCTGAACAAGGTG

FPPEVAVFEPSEAEISHT QK

TTCCCACCCGAGGTCGCTGTTTTGAGCCATCAGAAGCAGAGATCTCCCACACCCAAAAG

A T L V C L A T G F F P D H V E L S W W GCCACACTGGTGGCCACAGGCTTCTTCCCTGACCACGTGGAGCTGAGCTGGTGG

V N G K E V H S G V S Q D P Q P L K E Q GTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCCAGGACCCGCAGCCCCTCAAGGAGCAG

PALNDSRYSLSSRLRVSATF

CCCGCCCTCAATGACTCCAGATACAGCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCTTC

W Q N P R N H F R C Q V Q F Y G L S E N TGGCAGAACCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGGAGAAT

D E W T Q D R A K P V T Q I V S A E A W GACGACTGGACCCAGGATAGGGCCAAACCTGTCACCCAGATCGTCAGCGCCGAGGCCTGG

<TCR beta linker c-fos>

G R A D P G G L T D T L Q A E T D Q L E GGTAGAGCAGACCCCGGGGGTCTGACTGATACACTCCAAGCGGAGACAGATCAACTTGAA

D K K S A L Q T E I A N L L K E K E K L GACAAGAAGTCTGCGTTGCAGACCGAGATTGCCAATCTACTGAAAGAGAAGAAAAACTA

E F I L A A Y *
GAGTTCATCCTGGCAGCTTACTAG

Figure 14

TCR alfa>

M K Q E V T Q I P A A L S V P E G E N L ATGAAACAAGAAGTTACACAGATTCCTGCAGCTCTGAGTGTCCCAGAAGGAGAAAACTTG

V L N C S F T D S A I Y N L Q W F R Q D GTTCTCAACTGCAGTTTCACTGATAGCGCTATTTACAACCTCCAGTGGTTTAGGCAGGAC

G R L N A S L D K S S G R S T L Y I A A GGAAGACTTAATGCCTCGCTGGATAAATCATCAGGACGTAGTACTTTATACATTGCAGCT

S Q P G D S A T Y L C A V T N F N K F Y TCTCAGCCTGGTGACCACCTACCTCTGTGCTGTGACCAACTTCAACAAATTTTAC

F G S G T K L N V K P N I Q N P D P A V
TTTGGATCTGGGACCAAACTCAATGTAAAACCAAATATCCAGAACCCTGACCCTGCCGTG

S Q T N V S Q S K D S D V Y I T D K T V
TCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAACTGTG

L D M R S M D F K S N S A V A W S N K S CTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCT

<TCR alfa <u>linker</u> c-jun>

S P E S S P G G R I A R L E E K V K T L AGCCCAGAAAAGTTCCcccgggGGTAGAATCGCCCGGCTGGAGAAAAAGTGAAAAACCTTG

K A Q N S E L A S T A N M L R E Q V A Q AAAGCTCAGAACTCGGAGCTGGCGTCCACGGCCAACATGCTCAGGGAACAGGTGGCACAG

L K Q K V M N Y *
CTTAAACAGAAAGTCATGAACTACTAG

Figure 15

TCR beta>

M K A G V T Q T P R Y L I K T R G Q Q V ATGAAAGCTGGAGTTACTCAAACTCCAAGATATCTGATCAAAACGAGGACAGCAAGTG

T L S C S P I S G H R S V S W Y Q Q T P ACACTGAGCTGCTCCCCTATCTCTGGGCATAGGAGTGTATCCTGGTACCAACAGACCCCA

F P G R F S G R Q F S N S R S E M N V S
TTCCCTGGTCGATTCTCAGGGCGCCAGTTCTCTAACTCTCGCTCTGAGATGAATGTGAGC

T L E L G D S A L Y L C A S S F D S G N ACCTTGGAGCTGGGGGACTCGGCCCTTTATCTTTGCGCCAGCAGCTTCGACAGCGGGAAT

S P L H F G N G T R L T V T E D L N K V TCACCCCTCCACTTTGGGAACGGGACCAGGCTCACTGTGACAGAGGACCTGAACAAGGTG

F P P E V A V F E P S E A E I S H T Q K TTCCCACCCGAGGTCGCTGTGTTTGAGCCATCAGAAGGAGATCTCCCACACCCAAAAG

A T L V C L A T G F F P D H V E L S W W GCCACACTGGTGGCCACAGGCTTCTTCCCTGACCACGTGGAGCTGAGCTGGTGG

V N G K E V H S G V S Q D P Q P L K E Q GTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCCAGGACCCGCAGCCCCTCAAGGAGCAG

PALNDSRYSLSSRLRVSATFCCCGCCTCAATGACTCCAGATACAGCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCTTC

W Q N P R N H F R C Q V Q F Y G L S E N TGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGGAGAAT

<TCR beta <u>linker</u> c-fos>

G R A D P G G L T D T L Q A E T D Q L E GGTAGAGCAGACCCGggGGTCTGACTGATACACTCCAAGCGGAGACAGATCAACTTGAA

D K K S A L Q T E I A N L L K E K E K L GACAAGAAGACTTGCGTTGCAGACCGAGATTGCCAATCTACTGAAAGAGAAGAAAAACTA

E F I L A A Y *
GAGTTCATCCTGGCAGCTTACTAG

TCR alfa>

MQKEVEQNSGPLSVPEGAIA
atgCAGAAGGAAGTGCAGCAGAGCCATTGCC

S L N C T Y S D R G S Q S F F W Y R Q Y
TCTCTCAACTGCACTTACAGTCACCCAGGTTCCCAGTCCTTCTTCTGGTACAGACAATAT

S G K S P E L I M S I Y S N G D K E D G
TCTOCCAAAAGCCCTCAGTTCATAATGTCCATATACTCCAATGGTCACAAGACAACAACATCGA

R F T A Q L N K A S Q Y V S L L I R D S AGGITTACAGCACAGCICAATAAAGCCAGCCAGTATGITTCCTCCTCATCACACACCCC

Q P S D S A T Y L C A V T T D S W G K L CAGCCCAGGGATTCAGCCACCTACCTCTGTGCCGTTACAACTGACAGCTGGGGAAATTG

Q F G A G T Q V V V T P D I Q N P D P A CAGTITICAGAGGAGGGACCCAGTITICIGGICACCCCAGATATOCAGAACCCTGACCCTGCC

V Y Q L R D S K S S D K S V C L F T D F GIGTACCAGCICACACCICTAAATCCAGCACACICTGCTCACCCATTTT

DSQTNVSQSKDSDVYITDKT CATTCTCAAACAAATGIGICACAAAGTAAGGATTCTCATGIGIATATCACAGACAAAACT

V L D M R S M D F K S N S A V A W S N K GIGCTACACATCACGICTATGCACTTCAAGACAACACTGCTGTGGGCCTGCAGCAACAAA

<TCR alfa linker c-jun>
P S P E S S P G G R I A R L E E K V K T
CCCACCCACAAAGTTCCcccgggGGTACAATCCCCCCCCCCCCACAAAAAAGTGAAAACC

L K A Q N S E L A S T A N M L R E Q V A TTCAAACCTCAGAACTCGGAGCTGGGGTCCAGGGCCAACATGCTCAGGGAACAGGTGGCA

Q L K Q K V M N Y *
CACCITAAACAGAAGICATGAACIACTAG

T L Q C A Q D M N H E Y M S W Y R Q D P ACACTOCAGTGTGCCCAGCATATCAACCATCAATACATGTCCTGGTATCCACAACACCACA

SAAPSQTSVYFCASRPGLAG

GRPEQYFGPGTRLTVTEDLK

QKATLVCLATGFYPDHVELS CAAAAGGCCACACTGGTGGCCTGCCCCACAGGCTTCTACCCCCACCACGTGGAGCTGAGC

W W V N G K E V H S G V S T D P Q P L K TOGIOGGICAATGGCAGGGGCACAGGGCCTCAAG

EQPALNDSRYALLSSRLRVSA GAGCAGCCCGCCTCAATCACTCCACATACGCLCTCAGCCCCTCAGGCTCTCCGCCC

T F W Q N P R N H F R C Q V Q F Y G L S ACCTICIOSCAGAACOCCOSCAACCACTICOSCIGICAAGTICAAGTICTAOSSCICTCG

Continued

21/52 Figure 17 (continued)

Linker Biotinylation tag>
K L E F I L A A Y G S G G G L N D I F E
AAACTACAGTTCATCCTGCCACCTTACggatccGGTGGTGGTCTCAACCATATTTTTCAA

A Q K I E W H *
GCTCAGAAAATCGAATGGCATTAAGCTT

TCR alfa>

EGRISILNCODYTNSMFDYFL CAACCAACAATTICTATICTCAACTGTCACTATACTAACAGCATGTTTGATTATTTCCTA

W Y K K Y P A E G P T F L I S I S S I K
TCGTACAAAAATACOCTCCTCAACGTCCTACATTCCTCATATCTATAAGTTCCATTAAG

DKNEDGRFTVFLNKSAKHLS CATAAAAATCAACATGCAAGATCACTGTCTTCTTAAACAAAGTGCCAAGCACCTCTCT

L H I V P S Q P G D S A V Y F C A A M E CIGCACATIGIOCCCIOCCAGCCIOGACACICIGCAGIGIACTICIGIGCAGCAATGGAG

G A Q K L V F G Q G T R L T I N P N I Q

L F T D F D S Q T N V S Q S K D S D V Y
CTATICACCCATTICATICICAAACAAATGIGICACAAAGIAACGATTCICATGIGIAT

I T D K T V L D M R S M D F K S N S A V ATCACACACAAAACTGTGCTACACATGACGTCTATGCACTTCAAGACCAACAGTGCTGTG

A W S N K S D F A C A N A F N N S I I P GCCTGGAGCAACAACTGTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCA

<TCR alfa linker c-jun>

E K V K T L K A Q N S E L A S T A N M L GAAAAAGTGAAAACCTTGAAAGCTCAGAACTOOGAGCTGGAGCTGCACCACCACCATCCTC

R E Q V A Q L K Q K V M N Y *
ACCCAACACCTCAACCTCAACCTACTACTAC

TCR beta>

T L Q C A Q D M N H E Y M S W Y R Q D P ACACTOCAGTIGTOCCAGGATATGAACCATGAATACATGTOCTOGTATOGACAAGACCCA

V P N G Y N V S R S T T E D F P L R L L GICCCCAATGCTACAATGICTCCAGATCAACCACAGAGGATTICCCCCTCAGGCTGCTG

F Y E Q Y F G P G T R L T V T E D L K N
TTTTACGAGCAGTACTTCGGGGCGCACCACGCCTCACAGGACCTCAAAAAC

V F P P E V A V F E P S E A E I S H T Q GIGTICCCACCCGAGGICGCTGTGTTTGAGCCATCAGAAGCAGATCTCCCACACCCAA

KATLVCLATGFYPDHVELSW

W V N G K E V H S G V S T D P Q P L K E TECGTIGAATCOGAACCACACACCCCTCAACCAG

Q P A L N D S R Y $\underline{\textbf{A}}$ L S S R L R V S A T CAGCCCGCCCTCAATGACTCCAGATAC $\underline{\textbf{ct}}$ CTGAGCAGCCGCCTGAGCGTCTCGGCCACC

F W Q D P R N H F R C Q V Q F Y G L S E TICTGGCAGGACCCCCCCACCTTCCGCTGTCAAGTCCAGTTCTACGGCTCTCGCAG

N D E W T Q D R A K P V T Q I V S A E A AATGACGAGTGGACCCAGGATAGGGCCAAACCCGTCACCCAGATCGTCAGCGCCAGGCCC

Continued.....

Figure 19 (continued)

<TCR beta linker c-fos>
W G R A D P G G L T D T L Q A E T D Q L
TGGGGTAGAGCAGCACGGGGGGCACTGACTGATACACTCCAAGGGGAGACAGATCAACTT

E D K K S A L Q T E I A N L L K E K E K GAAGACAAGAAGTCTGCGTTGCAGACCCAGATTGCCAATCTGCTGAAAGAGAAGAAAAA

<u>linker</u> Biotinylation tag>
L E F I L A A Y G S G G L N D I F E A
CTACAGTTCATCCTGGCAGCTTACggatccGGTGGTGGTCTGAACGATATTTTTGAAGCT

Q K I E W H *
CAGAAAATOGAATOGCATTAAGCTT

TCR beta>

T L Q C A Q D M N H E Y M S W Y R Q D P ACACTGCAGTGTGCCCCAGGATATGAACCATGAATACATGTCCTGGTATGGACAAGACCCA

V P N G Y N V S R S T T E D F P L R L L GTCCCCAATGGCTACAATGTCTCCAGATCAACCACAGAGCATTTCCCCCTCAGGCTCCTG

G R P E Q Y F G P G T R L T V T E D L K G00000ACCACAGGGACCICAAA

Q K A T L V C L A T G F Y P D H V E L S CAAAAGGCCACACGGGGACCTGGACCTGACC

T F W Q D P R N H F R C Q V Q F Y G L S ACCTICIOGCAGGACCCCCGCAACCACTICCGCTGTCAAGTCCAGTTCTACCGCCTCTCCG

ENDEWTQDRAKPVTQIVSAE

Continued.....

Figure 20 (continued)

Linker Biotinylation tago
K L E F I L A A Y G S G G G L N D I F E
AAACTACAGTTCATCCTGGCAGCTTACggatccGGTGGTGGTCTGAACGATATTTTTGAA

A Q K I E W H *
GCTCAGAAAATCGAATGGCATTAAGCTT

. :

Linker<-> fos

P G G L T D T L Q A E T D Q 5'- ccc gag GGT CTG ACT GAT ACA CTC CAA GCG GAG ACA GAT CAA Xma I

L E D K K S A L Q T E I A N L CTT GAA GAC AAG AAG TCT GOG TTG CAG ACC GAG ATT GOC AAT CTA

<-lin
L K E K E K L E F I L A A Y G
CTG AAA GAG AAG GAA AAA CTA GAG TTC ATC CTG GCA GCT TAC gga
Bam</pre>

Ker-> <- biotinylation tag
S G G L N D I F E A Q K I E
tcc GGT GGT CTG AAC GAT ATT TTT GAA GCT CAG AAA ATC GAA
HI

W H *
TGG CAT <u>TAA GCT T</u> -3'
Hind III

Figure 22

A

Reverse primer:

 5^\prime--ACACAC GGA TCC GTA AGC TGC GAC GAT GAA CTC GAT TTT CTT- 3^\prime

Bam HI

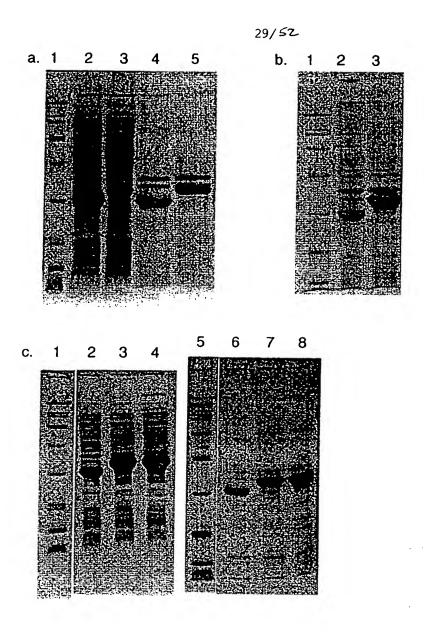


Figure 23

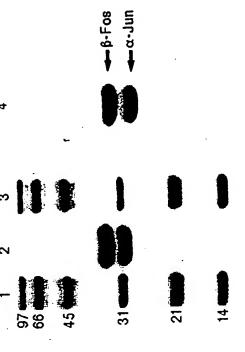


Figure 25.

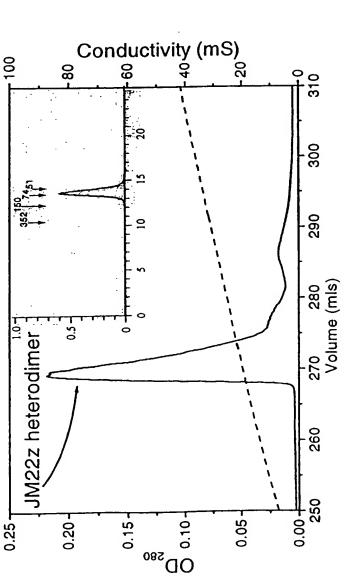
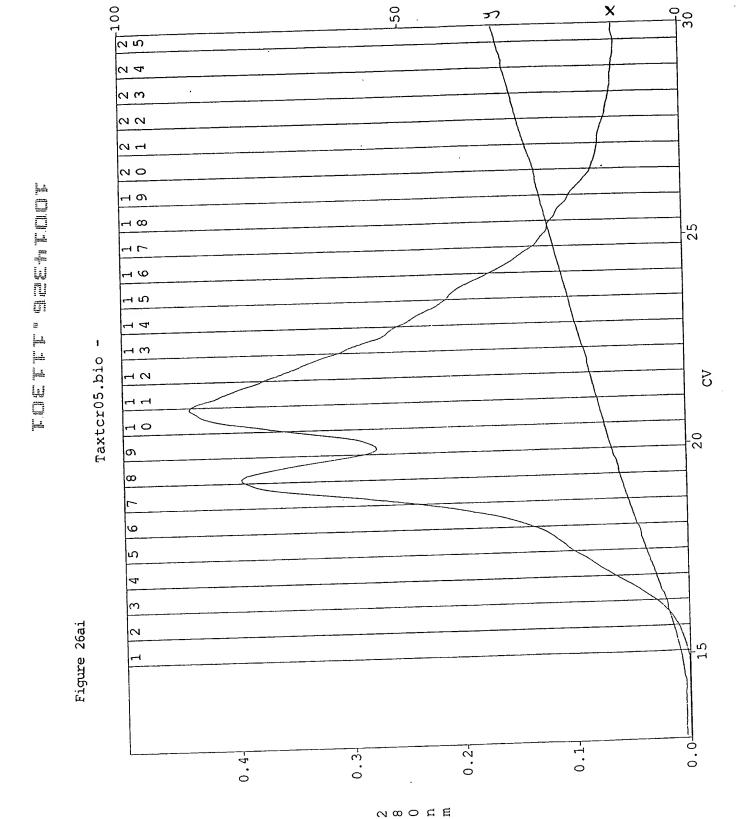
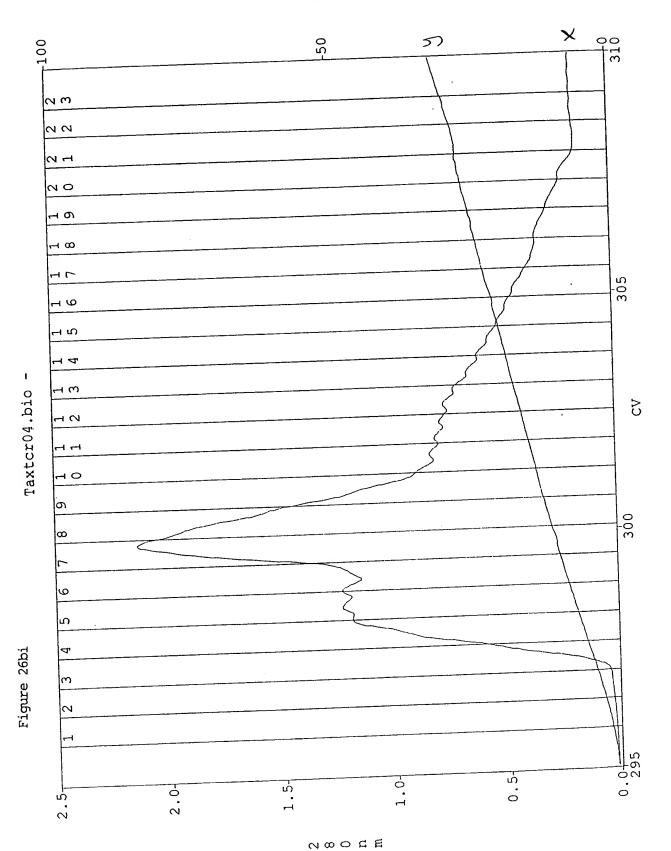


Figure 24.



E S



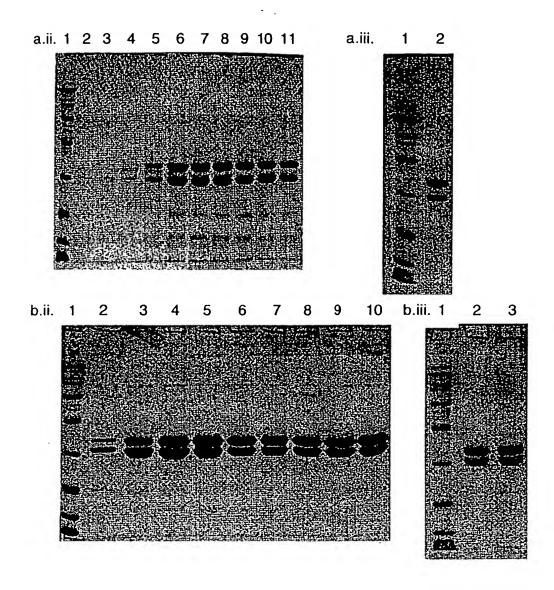
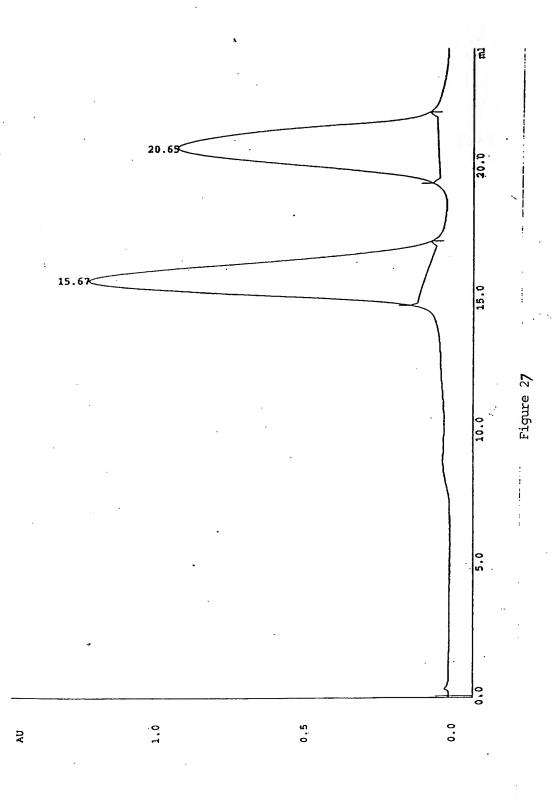


Figure 26



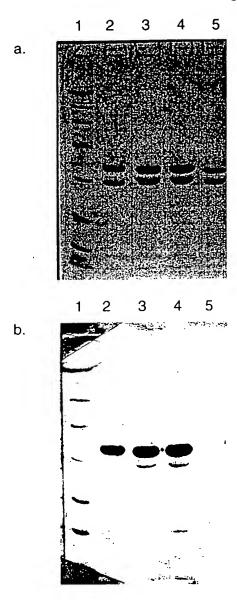


Figure 28

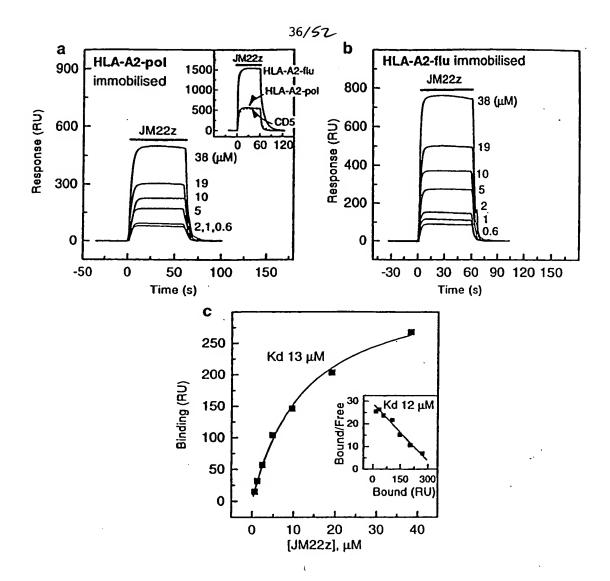


Figure 29

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Figure 31

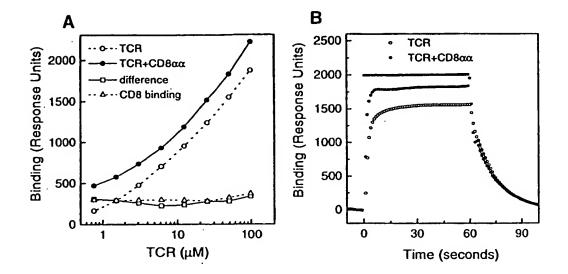


FIGURE 32

TCR alfa>

M Q L L E Q S P Q F L S I Q E G E N L T ATGCAaCTaCTaGAaCAaAGtCCTCAGTTTCTAAGCATCCAAGAGGGAGAAAATCTCACT

E G P V L L V T V V T G G E V K K L K R GAAGGTCCTGTCCTCCTGGTGACAGTAGTTACGGGTGGAGAAGTGAAGAAGCTGAAGAGA

L T F Q F G D A R K D S S L H I T A A Q CTAACCTTTCAGTTTGGTGATGCAAGAAAGGACAGTTCTCTCCACATCACTGCGGCCCAG

G K G T K L S V K P N I Q N P D P A V Y
GGAAAAGGCACTAAACTCTCTGTTAAACCAAATATCCAGAACCCTGACCCTGCCGTGTAC

Q L R D S K S S D K S V C L F T D F D S CAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTGATTCT

Q T N V S Q S K D S D V Y I T D K T V L CAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAACTGTGCTA

D M R S M D F K S N S A V A W S N K S D GACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGAC

F A C A N A F N N S I I P E D T F F P S
TTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGC

<TCR alfa linker c-jun>

P E S S P G G R I A R L E E K V K T L K
CCAGAAAGTTCCcccgggGGTAGAATCGCCCGGCTGGAGGAAAAAGTGAAAACCTTGAAA

A Q N S E L A S T A N M L R E Q V A Q L GCTCAGAACTCGGAGCTGGCGTCCACGGCCAACATGCTCAGGGAACAGGTGGCACAGCTT

K Q K V M N Y *
AAACAGAAAGTCATGAACTACTAG

FIGURE 33

	T	CR	be	t	а	>
--	---	----	----	---	---	---

V T L S C E Q N L N H D A M Y W Y R Q D GTGACCCTGAGTTGTGAACAGAATTTGAACCACGATGCCATGTACTGGTACCGACAGGAC

P G Q G L R L I Y Y S Q I V N D F Q K G CCAGGGCAAGGGCTGAGATTGATCTACTACTCACAGATAGTAAATGACTTTCAGAAAGGA

D I A E G Y S V S R E K K E S F P L T V GATATAGCTGAAGGGTACAGCGTCTCTCGGGAGAAGAAGAATCCTTTCCTCTCACTGTG

T S A Q K N P T A F Y L C A S S S R S S ACATCGGCCCAAAAGAACCCGACAGCTTTCTATCTCTGTGCCAGTAGTTCGAGGAGCTCC

Y E Q Y F G P G T R L T V T E D L K N V TACGAGCAGTACTTCGGGCCGGGCACCAGGCTCACGGTCACAGAGGACCTGAAAAACGTT

F P P E V A V F E P S E A E I S H T Q K
TTCCCACCCGAGGTCGCTGTTTTGAACCATCAGAAGCAGAGATCTCCCACACCCAAAAG

A T L V C L A T G F Y P D H V E L S W W
GCCACACTGGTGTGCCTGGCCACAGGCTTCTACCCCGACCACGTGGAGCTGAGCTGGTGG

V N G K E V H S G V S T D P Q P L K E Q GTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACAGACCCGCAGCCCCTCAAGGAGCAG

P A L N D S R Y S L S S R L R V S A T F

CCCGCCTCAATGACTCCAGATACTCCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCTTC

W Q N P R N H F R C Q V Q F Y G L S E N
TGGCAGAACCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGGAGAAT

D E W T Q D R A K P V T Q I V S A E A W
GACGAGTGGACCCAGATAGGGCCAAACCTGTCACCCAGATCGTCAGCGCCGAGGCCTGG

<TCR beta linker c-fos>

G R A D P G G L T D T L Q A E T D Q L E GGTAGAGCAGACCCCgggGGTCTGACTGATACACTCCAAGCGGAGACAGATCAACTTGAA

D K K S A L Q T E I A N L L K E K E K L GACAAGAAGTCTGCGTTGCAGACCGAGATTGCCAATCTACTGAAAGAGAAAAGCTA

E F I L A A Y *
GAGTTCATCCTGGCAGCTTACTAG

FIGURE 34

TCR beta>

V T L S C E Q N L N H D A M Y W Y R Q D GTGACCCTGAGTTGTGAACAGAATTTGAACCACGATGCCATGTACTGGTACCGACAGGAC

D I A E G Y S V S R E K K E S F P L T V
GATATAGCTGAAGGGTACAGCGTCTCTCGGGAGAAGAAGGAATCCTTTCCTCTCACTGTG

T S A Q K N P T A F Y L C A S S S R S S ACATCGGCCCAAAAGAACCCGACAGCTTTCTATCTCTGTGCCAGTAGTTCGAGGAGCTCC

F P P E V A V F E P S E A E I S H T Q K
TTCCCACCCGAGGTCGCTGTTTGAACCATCAGAAGCAGAGATCTCCCACACCCAAAAG

A T L V C L A T G F Y P D H V E L S W W GCCACACTGGTGTGCCTGGCCACAGGCTTCTACCCCGACCACGTGGAGCTGAGCTGGTGG

V N G K E V H S G V S T D P Q P L K E Q GTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACAGACCCGCAGCCCCTCAAGGAGCAG

P A L N D S R Y \underline{s} L S S R L R V S A T F CCCGCCCTCAATGACTCCAGATACTCCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCTTC

W Q N P R N H F R C Q V Q F Y G L S E N
TGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGGAGAAT

D E W T Q D R A K P V T Q I V S A E A W GACGAGTGGACCCAGGATAGGGCCAAACCTGTCACCCAGATCGTCAGCGCCGAGGCCTGG

<TCR beta <pre>linker c-fos>

G R A D P G G L T D T L Q A E T D Q L E GGTAGAGCAGACCCCgggGGTCTGACTGATACACTCCAAGCGGAGACAGATCAACTTGAA

D K K S A L Q T E I A N L L K E K E K L GACAAGAAGTCTGCGTTGCAGACCGAGATTGCCAATCTACTGAAAGAGAAAAACTA

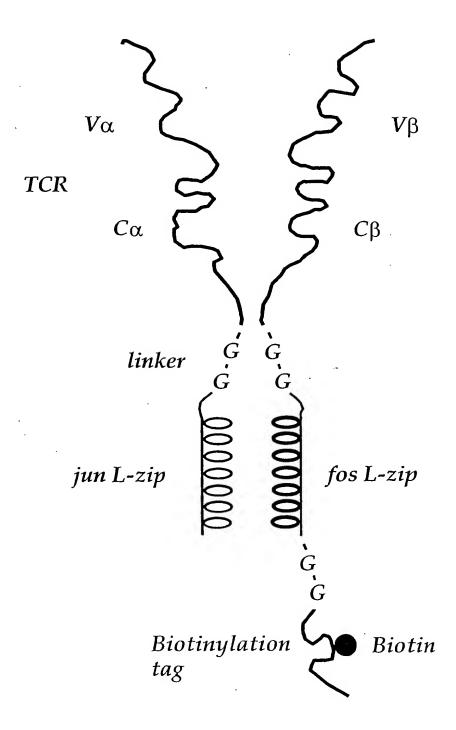
linker Biotinylation tag>

E F I L A A Y G S G G G L N D I F E A Q GAGTTCATCCTGGCAGCTTACggatccGGTGGTGGTCTGAACGATATTTTTGAAGCTCAG

K I E W H *
AAAATCGAATGGCATTAA

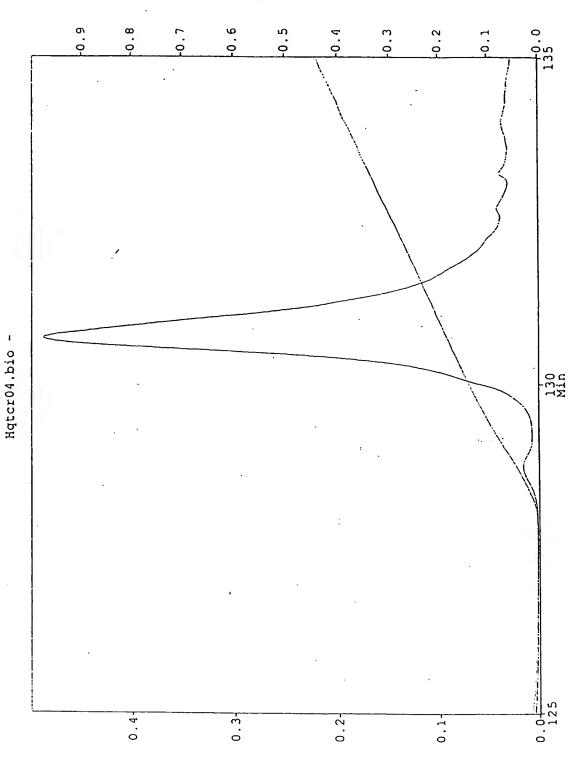
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FIGURE 35

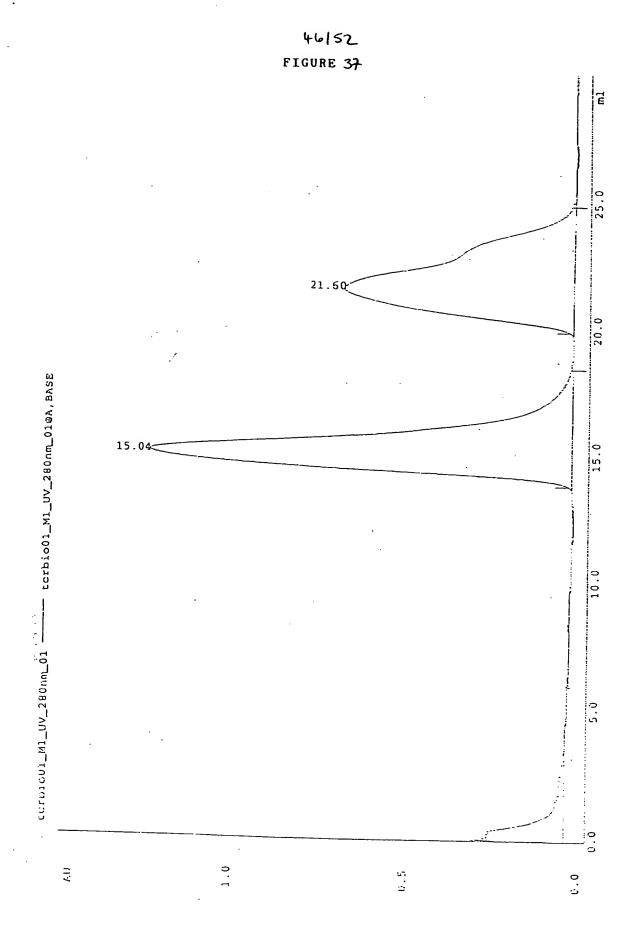


45|52 FIGURE *3*6

. 790 c E



000 C E



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FIGURE 38

TCR alfa> M Q K E V E Q N S G P L S V P E G A I A atgCAGAAGGAGCTGGAGCAGAACTCTGGACCCCTCAGTGTTCCAGAGGGAGCCATTGCC $\label{eq:cagactaga}$

S L N C T Y S D R G S Q S F F W Y R Q Y TCTCTCAACTGCACTTACAGTGACCGAGGTTCCCAGTCCTTCTTCTGGTACAGACAATAT

S G K S P E L I M S I Y S N G D K E D G TCTGGGAAAAGCCCTGAGTTGATAATGTCCATATACTCCAATGGTGACAAAGAAGATGGA

R F T A Q L N K A S Q Y V S L L I R D S AGGTTTACAGCACAGCTCAATAAAGCCAGCCAGTATGTTTCTCTGCTCATCAGAGACTCC

Q P S D S A T Y L C A V T T D S W G K L

CAGCCCAGTGATTCAGCCACCTACCTCTGTGCCGTTACAACTGACAGCTGGGGGAAATTG

 ${\tt Q}$ F G A G T Q V V V T P D I Q N P D P A CAGTTTGGAGCAGGGACCCAGGTTGTGGTCACCCCAGATATCCAGAACCCTGACCCTGCC

D S Q T N V S Q S K D S D V Y I T D K T GATTCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAACT

V L D M R S M D F K S N S A V A W S N K

GTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAA

S D F A C A N A F N N S I I P E D T F F TCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCTTC

<TCR alfa <u>linker</u> c-jun>
P S P E S S P G G R I A R L E E K V K T
CCCAGCCCAGAAAGTTCCcccgggGGTAGAATCGCCCGGCTGGAGGAAAAAGTGAAAACC

L K A Q N S E L A S T A N M L R E Q V A

TTGA-AGCTCAGAACTCGGAGCTGGCGTCCACGGCCAACATGCTCAGGGAACAGGTGGCA

Q L K Q K V M N Y * CAGCTTAAACAGAAAGTCATGAACTACTAG

FIGURE 39

- T L Q C A Q D M N H E Y M S W Y R Q D P ACACTGCAGTGTGCCCAGGATATGAACCATGAATACATGTCCTGGTATCGACAAGACCCA
- G M G L R L I H Y S V G A G I T D Q G E GGCATGGGGCTGAGGCTGATTCATTACTCAGTTGGTGCTGGTATCACTGACCAAGGAGAA
- $\begin{array}{cccccc} V & P & N & G & Y & N & V & S & R & S & T & T & E & D & F & P & L & R & L \\ GTCCCCAATGGCTACAATGTCTCCAGATCAACCACAGAGGATTTCCCGCTCAGGCTGCTG \\ \end{array}$
- S A A P S Q T S V Y F C A S R P G L A G TCGGCTGCTCCCAGACATCTGTGTACTTCTGTGCCAGCAGGCCGGGACTAGCGGGA
- G R P E Q Y F G P G T R L T V T E D L K GGGCGACCAGAGCAGTACTTCGGGCCGGGCACCAGGCTCACGGTCACAGAGGACCTGAAA
- N V F P P E V A V F E P S E A E I S H T AACGTGTTCCCACCCGAGGTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCCACACC
- Q K A T L V C L A T G F Y P D H V E L S CAAAAGGCCACACTGGTGTGCCTGGCCACAGGCTTCTACCCCGACCACGTGGAGCTGAGC
- W W V N G K E V H S G V S T D P Q P L K
 TGGTGGGTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACAGACCCGCAGCCCCTCAAG
- T F W Q N P R N H F R C Q V Q F Y G L S ACCTTCTGGCAGAACCCCGGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCG
- E N D E W T Q D R A K P V T Q I V S A E GAGAATGACGAGTGGACCCAGGATAGGGCCAAACCTGTCACCCAGATCGTCAGCGCCGAG
- <TCR beta linker c-fos>
 A W G R A D P G G L T D T L Q A E T D Q
 GCCTGGGGTAGAGCAGACCCCqqqGGTCTGACTGATACACTCCAAGCGGAGACAGATCAA

L E D K K S A L Q T E I A N L L K E K E CTTGAAGACAAGAAGTCTGCGTTGCAGACCGAGATTGCCAATCTACTGAAAGAAGAAGAA

 $\frac{linker}{\text{G} \text{ S} \text{ G} \text{ G} \text{ L} \text{ N} \text{ D} \text{ I} \text{ F} \text{ E}} \\ \text{AAACTAGAGTTCATCCTGGCAGCTTACggatccGGTGGTGGTCTGAACGATATTTTTGAA} \\$

A Q K I E W H *
GCTCAGAAAATCGAATGGCATTAAGCTT

FIGURE 40

TCR alfa>
M Q Q K N D D Q Q V K Q N S P S L S V Q
atgCAACAGAAGATGATGACCAGCAAGTTAAGCAAAATTCACCATCCCTGAGCGTCCAG

E G R I S I L'N C D Y T N S M F D Y F L GAAGGAAGAATTTCTATTCTGAACTGTGACTATACTAACAGCATGTTTGATTATTTCCTA

W Y K K Y P A E G P T F L I S I S S I K TGGTACAAAAAATACCCTGCTGAAGGTCCTACATTCCTGATATCTATAAGTTCCATTAAG

D K N E D G R F T V F L N K S A K H L S GATAAAAATGAAGATGGAAGATTCACTGTCTTCTTAAACAAAAGTGCCAAGCACCTCTCT

L H I V P S Q P G D S A V Y F C A A M E CTGCACATTGTGCCCTCCCAGCCTGGAGACTCTGCAGTGTACTTCTGTGCAGCAATGGAG

G A Q K L V F G Q G T R L T I N P N I Q GGAGCCCAGAAGCTGGTATTTGGCCAAGGAACCAGGCTGACTATCAACCCAAATATCCAG

L F T D F D S Q T N V S Q S K D S D V Y CTATTCACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTAT

I T D K T V L D M R S M D F K S N S A V ATCACAGACAAAACTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTG

A W S N K S D F A C A N A F N N S I I P GCCTGGAGCAACAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCA

E K V K T L K A Q N S E L A S T A N M L GAAAAAGTGAAAACCTTGAAAGCTCAGAACTCGGAGCTGGCGTCCACGGCCAACATGCTC

R E Q V A Q L K Q K V M N Y *
AGGGAACAGGTGGCACAGCTTAAACAGAAAGTCATGAACTACTAG

FIGURE 4

- TCR beta> M N A G V T Q T P K F Q V L K T G Q S M atgAACGCTGGTGTCACTCAGACCCCAAAATTCCAGGTCCTGAAGACAGGACAGAGCATG
- T L Q C A Q D M N H E Y M S W Y R Q D P ACACTGCAGTGTGCCCAGGATATGAACCATGAATACATGTCCTGGTATCGACAAGACCCA
- G M G L R L I H Y S V G A G I T D Q G E GGCATGGGGCTGAGGCTGATTCATTACTCAGTTGGTGCTGGTATCACTGACCAAGGAGAA
- V P N G Y N V S R S T T E D F P L R L L GTCCCCAATGGCTACAATGTCTCCAGATCAACCACAGAGGATTTCCCGCTCAGGCTGCTG
- S A A P S Q T S V Y F C A S S Y P G G G TCGGCTGCTCCCTCCCAGACATCTGTGTACTTCTGTGCCAGCAGTTACCaGGaGGGGGG
- F Y E Q Y F G P G T R L T V T E D L K N TTTTACGAGCAGTACTTCGGGCCGGGCACCAGGCTCACGGTCACAGAGGACCTGAAAAAC
- V F P P E V A V F E P S E A E I S H T Q GTGTTCCCACCCGAGGTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCCACACCCAA
- K A T L V C L A T G F Y P D H V E L S W AAGGCCACACTGGTGTGCCTGGCCACAGGCTTCTACCCCGACCACGTGGAGCTGAGCTGG
- W V N G K E V H S G V S T D P Q P L K E TGGGTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACAGACCCGCAGCCCCTCAAGGAG
- Q P A L N D S R Y A L S S R L R V S A T CAGCCCGCCCTCAATGACTCCAGATACgetCTGAGCAGCCGCCTGAGGGTCTCGGCCACC
- F W Q D P R N H F R C Q V Q F Y G L S E TTCTGGCAGGACCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGGAG
- N D E W T Q D R A K P V T Q I V S A E A AATGACGAGTGGACCCAGGATAGGGCCAAACCCGTCACCCAGATCGTCAGCGCCGAGGCC

E D K K S A L Q T E I A N L L K E K E K GAAGACAAGAAGTCTGCGTTGCAGACCGAGATTGCCAATCTACTGAAAGAGAAAAAA

L E F I L A A Y G S G G G L N D I F E A CTAGAGTTCATCCTGGCAGCTTACGGATCGTGGTGGTGGTCTGAACGATATTTTTGAAGCT

Q K I E W H *
CAGAAAATCGAATGGCATTAAGCTT